



EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2015. Scientific Opinion on the substantiation of a health claim related to -galactosidase from *Kluyveromyces lactis* in Colief® and a reduction of gastrointestinal discomfort pursuant to Article 14 of Regulation (EC) No 1924/2006

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SCIENTIFIC OPINION

Scientific Opinion on the substantiation of a health claim related to β -galactosidase from *Kluyveromyces lactis* in Colief[®] and a reduction of gastrointestinal discomfort pursuant to Article 14 of Regulation (EC) No 1924/2006¹

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)^{2,3}

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following an application from Cross Vetpharm Group UK Ltd, submitted for authorisation of a health claim pursuant to Article 14 of Regulation (EC) No 1924/2006 via the Competent Authority of the United Kingdom, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to β -galactosidase from *Kluyveromyces lactis* in Colief[®] and a reduction of gastrointestinal discomfort. The Panel considers that the food is sufficiently characterised in relation to the claimed effect. A reduction of gastrointestinal discomfort is a beneficial physiological effect for infants and young children. The applicant provided two human intervention studies for the substantiation of the health claim. No conclusions could be drawn from one of the two studies for the scientific substantiation of the claim, as the information provided in the publication and that supplied later by the applicant was inadequate to allow a scientific evaluation. The second study with methodological limitations showed an effect of the food on crying time in infants fed exclusively with milk. This study also provided some evidence for the proposed mechanism by which β -galactosidase could exert the claimed effect. In weighing the evidence, the Panel took into account that one study with methodological limitations showed an effect of β -galactosidase from *Kluyveromyces lactis* in Colief[®] on infant crying time, that no other human studies in which these results have been replicated were provided, and that there was some evidence for a mechanism by which the food could exert the claimed effect. The Panel concludes that the evidence provided is insufficient to establish a cause and effect relationship between the consumption of β -galactosidase from *Kluyveromyces lactis* in Colief[®] and a reduction of gastrointestinal discomfort.

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KEY WORDS

β -galactosidase, *Kluyveromyces lactis*, gastrointestinal discomfort, children, health claims

¹ On request from the Competent Authority of the United Kingdom following an application by Cross Vetpharm Group UK Ltd, Question No EFSA-Q-2014-00404, adopted on 30 June 2015.

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SUMMARY

Following an application from Cross Vetpharm Group UK Ltd, submitted for authorisation of a health claim pursuant to Article 14 of Regulation (EC) No 1924/2006 via the Competent Authority of the United Kingdom, the European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to β -galactosidase from *Kluyveromyces lactis* in Colief[®] and a reduction of gastrointestinal discomfort.

The scope of the application was proposed to fall under a health claim referring to children's development and health.

The food that is the subject of the health claim is 'Colief[®]/lactase enzyme produced by controlled fermentation of *Kluyveromyces lactis*'. Ingested lactose is hydrolysed by lactase, an enzyme of the microvillus membrane of the enterocytes, into its components, glucose and galactose. The exogenous enzymes which are used to hydrolyse lactose are microbial β -galactosidases. The β -galactosidase which is the subject of the health claim is derived from the yeast *Kluyveromyces lactis*. The Panel considers that the food, β -galactosidase from *Kluyveromyces lactis* in Colief[®], which is the subject of the health claim, is sufficiently characterised in relation to the claimed effect.

The claimed effect proposed by the applicant is 'reduces the lactose load of the infant's feed and improves the consequences of lactose maldigestion in colicky infants unable to effectively digest all the lactose in their feed'. The target population proposed by the applicant is 'infants prior to weaning, aged up to 4–5 months with signs of colic, i.e. excessive crying and associated features of lactose overload'. Upon a request for clarification, the applicant indicated that 'reduction of gastrointestinal discomfort' was the claimed effect and that infant crying time was the outcome measure. The Panel considers that a reduction of gastrointestinal discomfort is a beneficial physiological effect for infants and young children.

The applicant provided two human intervention studies for the substantiation of the health claim.

In a randomised, double-blind, cross-over study, 14 infants were fed, for one week, formula to which β -galactosidase from *Kluyveromyces lactis* or placebo was added, with a two-day wash-out period in between. Infants were included in the study if they exhibited 'full force' crying for three or more hours a day for three or more days a week. Crying time was assessed using parents' diaries. Upon repeated requests from EFSA for more details on the statistical analysis, the applicant indicated that it was not possible to retrieve any statistical analysis plan or full study report 18 years after publication of the study. The Panel notes that the information provided in the publication and that supplied by the applicant in reply to requests for additional information was inadequate to allow a scientific evaluation. The Panel considers that no conclusions can be drawn from this study for the scientific substantiation of the claim.

Another randomised, double-blind, cross-over study included 53 infants. The two cross-over periods lasted for 10 days each with a 5-day wash-out period in between. In order to be eligible for the study, infants had to exhibit for at least 14 days 'full force' crying for more than three hours per day for three days or more per week, together with spasm, lower limb flexure and diarrhoea. Formula-fed infants were given formula to which β -galactosidase preparation was added. For breast-fed infants, mothers expressed the fore-milk into a teaspoon to which β -galactosidase preparation was added. During the control period a placebo was used.

Outcome measures were crying time and breath hydrogen concentration. Responders were defined *a priori* as those achieving a reduction of at least 45 % in both crying time and breath hydrogen concentrations.

The outcome variables were analysed only in the population of completers (n = 46 for crying time; n = 34 for breath hydrogen concentrations) and for the per-protocol (PP) population of compliers (n = 32 for crying time; n = 25 for breath hydrogen concentrations) but not in the intention-to-treat population (i.e. all the infants randomised for the study). The Panel notes that only 47 % of the infants randomised completed the study as planned (PP population), and that the PP analysis is at risk of selection bias.

The responder analysis was restricted to a subset of 34 infants for whom data on both crying time and breath hydrogen concentrations were available. Of these, nine infants (26 %) were classified as responders following consumption of β -galactosidase, while no infant fulfilled the responder criteria when consuming placebo. The difference in the numbers of responders to β -galactosidase and to placebo, respectively, was statistically significant ($p = 0.002$). The applicant also provided information on responders considering only crying time for the completers' population (n = 46) and the PP population (n = 32). In the completers' population, 16 infants were classified as responders following consumption of β -galactosidase, while three infants qualified as responders when consuming placebo. In the PP population, 15 infants were classified as responders following consumption of β -galactosidase, while two infants qualified as responders when consuming placebo.

Statistical analyses were also performed for absolute differences in crying time following consumption of β -galactosidase and placebo. In the population of completers (n = 46), there were no statistically significant differences in crying time between β -galactosidase and placebo. In the PP population (n = 32), crying time was significantly lower during the consumption of β -galactosidase (median: 520 minutes) than for placebo (median: 872.5 minutes; $p = 0.0052$).

The areas under the curve for breath hydrogen concentrations were significantly lower during consumption of β -galactosidase than for placebo in both the completers' population (6.0 ppm vs. 11.5 ppm; $p < 0.0001$) and the PP population (6.0 ppm vs. 9.5 ppm; $p < 0.0052$).

The Panel considers that this study with methodological limitations (no intention-to-treat analysis, PP analysis at risk of bias) shows an effect of β -galactosidase from *Kluyveromyces lactis* in Colief[®] on crying time in infants fed exclusively with milk. The Panel considers that this study also provides some evidence for the proposed mechanism by which β -galactosidase could exert the claimed effect.

In weighing the evidence, the Panel took into account that one study with methodological limitations showed an effect of β -galactosidase from *Kluyveromyces lactis* in Colief[®] on infant crying time, that no other human studies in which these results have been replicated were provided, and that there is some evidence for a mechanism by which the food could exert the claimed effect.

The Panel concludes that the evidence provided is insufficient to establish a cause and effect relationship between the consumption of β -galactosidase from *Kluyveromyces lactis* in Colief[®] and a reduction of gastrointestinal discomfort.

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BACKGROUND

Regulation (EC) No 1924/2006⁴ harmonises the provisions that relate to nutrition and health claims, and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of this Regulation, are authorised in accordance with this Regulation, and are included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Articles 14 to 17 of this Regulation lay down provisions for the authorisation and subsequent inclusion of reduction of disease risk claims and claims referring to children's development and health in a Community list of permitted claims.

According to Article 15 of this Regulation, an application for authorisation shall be submitted by the applicant to the national competent authority of a Member State, which will make the application and any supplementary information supplied by the applicant available to the European Food Safety Authority (EFSA).

STEPS TAKEN BY EFSA

- The application was received on 10/06/2014.
- The scope of the application was proposed to fall under a health claim referring to children's development and health.
- On 11/07/2014, during the validation process of the application, EFSA sent a request to the applicant to provide missing information.
- On 06/08/2014, EFSA received the applicant's reply.
- The scientific evaluation procedure started on 11/08/2014.
- On 19/09/2014, the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application and the scientific evaluation was suspended on 23/09/2014, in compliance with Article 16(1) of Regulation (EC) No 1924/2006.
- On 20/11/2014, EFSA received the applicant's reply and the scientific evaluation was restarted, in compliance with Article 16(1) of Regulation (EC) No 1924/2006.
- On 22/01/2015, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application and the scientific evaluation was suspended on 28/01/2015, in compliance with Article 16(1) of Regulation (EC) No 1924/2006.
- On 13/03/2015, EFSA received the applicant's reply and the scientific evaluation was restarted, in compliance with Article 16(1) of Regulation (EC) No 1924/2006.
- During its meeting on 30/06/2015, the NDA Panel, having evaluated the data submitted, adopted an opinion on the scientific substantiation of a health claim related to β -galactosidase from *Kluyveromyces lactis* in Colief® and a reduction of gastrointestinal discomfort.

TERMS OF REFERENCE

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16 of Regulation (EC) No 1924/2006. On the basis of that evaluation, EFSA will issue an

⁴ Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25.

opinion on the scientific substantiation of a health claim related to: β -galactosidase from *Kluyveromyces lactis* in Colief[®] and a reduction of gastrointestinal discomfort.

EFSA DISCLAIMER

The present opinion does not constitute, and cannot be construed as, an authorisation for the marketing of β -galactosidase from *Kluyveromyces lactis* in Colief[®], a positive assessment of its safety or a decision on whether β -galactosidase from *Kluyveromyces lactis* in Colief[®] is, or is not, classified as a foodstuff. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

It should also be highlighted that the scope, the proposed wording of the claim and the conditions of use as proposed by the applicant may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 17 of Regulation (EC) No 1924/2006.

INFORMATION PROVIDED BY THE APPLICANT

Applicant's name and address

Cross Vetpharm Group UK, Ltd, Bryn Cefni Industrial Park, Llangefni, Anglesey LL77 7XA, Wales, United Kingdom.

Food/constituent as stated by the applicant

According to the applicant, the food which is the subject of the claim is 'Colief[®]/lactase enzyme produced by controlled fermentation of *Kluyveromyces lactis*'.

Health relationship as claimed by the applicant

According to the applicant, the health relationship relates to 'the alleviation of the clinical features of secondary lactose maldigestion due to lactose overload [in infants]. Lactase enzyme added to the milk feed prior to ingestion, catalyses hydrolysis of the disaccharide lactose into molar equivalents of the monosaccharides glucose and galactose which the infant can absorb. This reduces the amount of lactose entering the colon and undergoing microbial fermentation, which lessens the adverse effects in susceptible infants. [...] The outcome measures used to assess the claimed effect in infants are measurement of breath hydrogen (objective measure) and infant crying time (quasi-objective measure). [...] The rationale for using crying time as a marker is that excessive crying time is a diagnostic feature of infantile colic, of which lactose overload is one possible cause.'

Wording of the health claim as proposed by the applicant

The applicant has proposed the following wording for the health claim: 'Colief[®]/lactase enzyme reduces the lactose load of the infant's feed and improves the consequences of lactose maldigestion in colicky infants unable to effectively digest all the lactose in their feed.'

Specific conditions of use as proposed by the applicant

According to the applicant, 'for milk for imminent use the quantity required is four drops of the lactase enzyme added to each feed (expressed breast milk, reconstituted formula or ready-to-use formula). The drops are added 30 minutes before the commencement of feeding. For feeds prepared at least four hours in advance of feeding, two drops are used. It is estimated that this dosing regimen converts at least 60 % of the available lactose per feed to molar equivalents of glucose and galactose. For breast feeding, four drops are added to a few tablespoons of expressed breast milk and given to the infant by sterilised spoon, following which breastfeeding is initiated as normal.' The applicant specified that the target population for the claim is 'infants prior to weaning, aged up to 4–5 months with signs of colic, i.e. excessive crying and associated features of lactose overload (spasm, lower limb flexure and diarrhoea)'.

ASSESSMENT

1. Characterisation of the food/constituent

The food that is the subject of the health claim is 'Colief[®]/lactase enzyme produced by controlled fermentation of *Kluyveromyces lactis*'.

Ingested lactose is hydrolysed by lactase, an enzyme of the microvillus membrane of the enterocytes, into its components, glucose and galactose. The exogenous enzymes which are used to hydrolyse lactose are microbial β -galactosidases mainly derived from *Kluyveromyces lactis*, *Kluyveromyces fragilis* and *Aspergillus oryzae* (EFSA NDA Panel, 2010).

The β -galactosidase which is the subject of the health claim is derived from the yeast *Kluyveromyces lactis*.

According to the applicant, the β -galactosidase which is produced by *Kluyveromyces lactis* is stable and active over a pH range of 6–8.

Information pertaining to the stability of the commercial product containing β -galactosidase from *Kluyveromyces lactis* produced by the applicant was provided. The lactase enzyme activity in the commercial product is 4 500 Food Chemical Codex Neutral Lactase Units (FCC NLU) per gram. It is supplied in liquid form.

The Panel notes that β -galactosidase from *Kluyveromyces lactis* is a well-characterised enzyme and that its activity can be measured in foods by established methods.

The Panel considers that the food, β -galactosidase from *Kluyveromyces lactis* in Colief[®], which is the subject of the health claim, is sufficiently characterised in relation to the claimed effect.

2. Relevance of the claimed effect to human health

The claimed effect proposed by the applicant is ‘reduces the lactose load of the infant’s feed and improves the consequences of lactose maldigestion in colicky infants unable to effectively digest all the lactose in their feed’. The target population proposed by the applicant is ‘infants prior to weaning, aged up to 4–5 months with signs of colic, i.e. excessive crying and associated features of lactose overload (spasm, lower limb flexure and diarrhoea)’.

Upon a request for clarification, the applicant indicated that ‘reduction of gastrointestinal discomfort’ was the claimed effect and that infant crying time was the outcome measure.

Unexplained bouts of crying in young infants have been traditionally attributed to gastrointestinal disturbances and pain (Shamir et al., 2013). The term infant colic is commonly used to reflect this situation in young infants. Infant colic has been included in the list of childhood functional gastrointestinal disorders of the Rome III Coordinating Committee, with diagnostic criteria based on infant crying time and frequency (Hyman et al., 2006). The Panel considers that crying time can be used to assess gastrointestinal discomfort in infants diagnosed with infant colic.

The Panel considers that a reduction of gastrointestinal discomfort is a beneficial physiological effect for infants and young children.

3. Scientific substantiation of the claimed effect

The applicant indicated that the lactase enzyme added to the milk feed prior to ingestion catalyses the hydrolysis of the disaccharide lactose into molar equivalents of the monosaccharides glucose and galactose, which infants can absorb, and that this would reduce the amount of lactose entering the colon and undergoing microbial fermentation, and thus gastrointestinal discomfort in susceptible infants. The applicant claims that breath hydrogen concentrations are an indirect measure of undigested carbohydrates, including lactose, in the gastrointestinal tract.

The applicant performed a literature search in Medline, Embase, Embase Alert and the Cochrane Library for publications in English using the following key words: ‘lactase’ AND ‘infant colic’ OR ‘crying’ OR ‘breath hydrogen’ AND ‘infant’. Additional searching was also done by hand. Studies were included if they were of a ‘sound design’ with a suitable control/treatment period and washout period, if they investigated the effect of lactase enzyme on indicators of ‘lactose overload’ in infants aged up to six months and prior to commencement of weaning, and if they included lactase enzyme added to milk (i.e. studies were excluded in cases in which lactase enzyme was given directly to infants before or following a milk feed).

The applicant provided two human intervention studies (Kearney et al., 1998; Kanabar et al., 2001) which assessed the effect of the food (i.e. β -galactosidase from *Kluyveromyces lactis* in Colief[®]) on infant crying time. One of the studies (Kanabar et al., 2001) also assessed the effect of Colief[®] on breath hydrogen concentrations, an indirect indicator of fermentation of undigested carbohydrates (i.e. mainly lactose in infants fed exclusively milk).

In a randomised, double-blind, cross-over study by Kearney et al. (1998), 14 infants (of whom 13 completed; completers' age and birth weight: 23–112 days, 2.9–4.7 kg) were fed parent-selected formula to which β -galactosidase from *Kluyveromyces lactis* or placebo (unspecified) was added 24 hours before feeding for one week, with a two-day wash-out period in between. Infants were included in the study if they exhibited 'full force' crying for three or more hours a day for three or more days a week.

In the original publication, the study was reported to have been carried out with 'Lactaid[®]' rather than with 'Colief[®]'. Upon request for clarification and specification of the β -galactosidase used in the study, the applicant provided a signed statement which indicated that subsequent to publication of the study the commercial name of the product was changed from Lactaid[®] to Colief[®] and that the two products are identical. The applicant also stated that the enzymatic activity of the lactase enzyme at the time of the study was the same as the enzymatic activity in the current product (i.e. Colief[®]) for which the health claim is made.

Following a request for clarification, the applicant indicated that the intended randomisation procedure (i.e. 6 blocks of 4 with 24 subjects in total) described in the publication could not be followed because of difficulties with recruitment. The applicant also stated that only 14 infants were randomised, that of these one infant was excluded from the analysis because 'his/her age was outside the range specified in the inclusion criteria' and that it has not been possible to retrieve any data for this excluded infant. The Panel notes that age was not indicated as an inclusion criterion in the publication and that no power calculations were provided.

Crying time was assessed using parents' diaries. In the publication it was reported that the analysis of crying time was based on a 'model for a two-period crossover trial with a covariate' (Jones and Kenward, 1989). Upon repeated requests from EFSA for more details on the statistical analysis, the applicant indicated that it was not possible to retrieve any statistical analysis plan or full study report 18 years after publication of the study.

The Panel notes that the information provided in the publication and that supplied by the applicant in reply to requests for additional information was inadequate to allow a scientific evaluation.

The Panel considers that no conclusions can be drawn from this study for the scientific substantiation of the claim.

The randomised, double-blind, cross-over study by Kanabar et al. (2001) included 53 infants aged between 3 and 13 weeks. The two cross-over periods lasted for 10 days each with a five-day washout period in between. In order to be eligible for the study, infants had to exhibit for at least 14 days 'full force' crying for more than three hours per day for three days or more per week, together with spasm, lower limb flexure and diarrhoea.

Formula-fed infants were given formula to which two drops of β -galactosidase preparation was added four hours before feeding. For breast-fed infants, mothers expressed the fore-milk into a teaspoon to which four drops of β -galactosidase were added. Mothers then breast-fed as usual, and gave the treated fore-milk to the infant at the end of the feed. During the control period, a placebo (heat-inactivated lactase) was used.

In the publication it was reported that the β -galactosidase used in the trial was derived from *Aspergillus*. Upon request for clarification on the food used in the study, the applicant provided

signed statements indicating that the applicant was the sponsor of the study and that the applicant's β -galactosidase product used in the study was sourced from *Kluyveromyces lactis* and not from *Aspergillus*. The Panel notes that an erratum which confirmed such information was published in 2007.

Compliance with the study protocol was assessed using returned bottles in which the study products had been distributed in a volume of 7 ml. Infants were considered to be non-compliant when the contents in the returned bottles exceeded 3.5 ml.

The Panel notes the poor reporting of the published study (Kanabar et al., 2001), which was communicated to the applicant in several requests for additional information. The description of the study methods and results which is given below summarises the information provided by the applicant in reply to EFSA's requests.

Outcome measures of the study were crying time and breath hydrogen concentration. Crying time was assessed using daily records filled in by the parents. Breath hydrogen was measured (using a portable hydrogen monitor) during the last two days at the end of each period. Measurements were performed before feeding, and at 10-minute intervals thereafter up to a maximum of 120 minutes or until breath hydrogen returned to the pre-feed baseline values. All readings over the pre-prandial baseline were summed to give an approximate area under the curve (AUC) value. Responders were defined *a priori* as those achieving a reduction of at least 45 % in both crying time and breath hydrogen concentrations. According to the applicant, the responder analysis was the pre-specified primary endpoint of the study. No reasons were provided for choosing a cut-off of 45 % reduction in both outcome variables.

The outcome variables were analysed only in the population of completers (n = 46 for crying time; n = 34 for breath hydrogen concentrations) and for the per-protocol (PP) population of compliers (n = 32 for crying time; n = 25 for breath hydrogen concentrations). The applicant claimed that no analyses could be conducted in the intention-to-treat (ITT) study population (all subjects randomised, n = 53). The Panel notes that only 47 % of the infants randomised completed the study as planned (PP population), and that the PP analysis is at risk of selection bias.

The responder analysis was restricted to a subset of 34 infants for whom data on both crying time and breath hydrogen concentrations were available. Of these, nine infants (26 %, 95 % binomial confidence interval: 12.9–44.4 %) were classified as responders following consumption of β -galactosidase, while no infant fulfilled the responder criteria when consuming placebo. The difference in the numbers of responders to β -galactosidase and to placebo, respectively, was statistically significant (p = 0.002, Fisher's exact test). In the PP population (n = 24), nine infants (38 %, 95 % binomial confidence interval: 18.8–59.4 %) were classified as responders. The applicant also provided information on responders considering only crying time for both the completers' population and the PP population. In the completers' population (n = 46), 16 infants were classified as responders following consumption of β -galactosidase, while 3 infants qualified as responders when consuming placebo. The difference in the numbers of responders (considering crying time only) to β -galactosidase and to placebo, respectively, was statistically significant (p = 0.0015, Fisher's exact test). In the PP population (n = 32), 15 infants were classified as responders following consumption of β -galactosidase, while 2 infants qualified as responders when consuming placebo.

Statistical analyses were also performed for absolute differences in crying time following consumption of β -galactosidase and placebo, respectively. As the data had a non-normal distribution, the non-parametric method proposed by Koch (1972) was applied, in order to examine the effects of treatment, period and order of the treatments. Results are given as medians and interquartile ranges (IQRs). In the population of completers (n = 46), there were no statistically significant differences in crying time between β -galactosidase (median, IQR: 657.5 minutes, 320–1 200 minutes) and placebo (median, IQR: 847.5 minutes, 515–1 515 minutes; p = 0.09). Results of the effect of period and

treatment order were not reported. In the PP population ($n = 32$), crying time was significantly lower during the consumption of β -galactosidase (median, IQR: 520 minutes, 305–1 192.5 minutes) than for placebo (median, IQR: 872.5 minutes, 595–1 585 minutes; $p = 0.0052$). The effects of period and treatment order were not statistically significant.

The AUC for breath hydrogen concentrations were significantly lower during consumption of β -galactosidase than for placebo in both the completers' population (median, IQR: 6.0 ppm (3.0–7.0 ppm) vs. 11.5 ppm (6.0–22.0 ppm); $p < 0.0001$) and the PP population (median, IQR: 6.0 ppm (4.0–7.0 ppm) vs. 9.5 ppm (5.5–23.0 ppm); $p < 0.0052$).

The Panel considers that this study with methodological limitations (no ITT analysis, PP analysis at risk of bias) shows an effect of β -galactosidase from *Kluyveromyces lactis* in Colief[®] on crying time in infants fed exclusively with milk. The Panel considers that this study also provides some evidence for the proposed mechanism by which β -galactosidase could exert the claimed effect.

In weighing the evidence, the Panel took into account that one study with methodological limitations showed an effect of β -galactosidase from *Kluyveromyces lactis* in Colief[®] on infant crying time, that no other human studies in which these results have been replicated were provided, and that there is some evidence for a mechanism by which the food could exert the claimed effect.

The Panel concludes that the evidence provided is insufficient to establish a cause and effect relationship between the consumption of β -galactosidase from *Kluyveromyces lactis* in Colief[®] and a reduction of gastrointestinal discomfort.

CONCLUSIONS

On the basis of the data presented, the Panel concludes that:

- The food, β -galactosidase from *Kluyveromyces lactis* in Colief[®], is sufficiently characterised in relation to the claimed effect.
- The claimed effect proposed by the applicant is 'reduces the lactose load of the infant's feed and improves the consequences of lactose maldigestion in colicky infants unable to effectively digest all the lactose in their feed'. The target population proposed by the applicant is 'infants prior to weaning, aged up to 4–5 months with signs of colic, i.e. excessive crying and associated features of lactose overload (spasm, lower limb flexure and diarrhoea)'. Upon a request for clarification, the applicant indicated that 'reduction of gastrointestinal discomfort' was the claimed effect and that infant crying time was the outcome measure. A reduction of gastrointestinal discomfort is a beneficial physiological effect for infants and young children.
- The evidence provided is insufficient to establish a cause and effect relationship between the consumption of β -galactosidase from *Kluyveromyces lactis* in Colief[®] and a reduction of gastrointestinal discomfort.

DOCUMENTATION PROVIDED TO EFSA

Health claim application on β -galactosidase from *Kluyveromyces lactis* and a reduction of gastrointestinal discomfort pursuant to Article 14 of Regulation (EC) No 1924/2006 (Claim serial No: 0417_UK). June 2014. Submitted by Cross Vetpharm Group UK, Ltd.

REFERENCES

- EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2010. Scientific Opinion on lactose thresholds in lactose intolerance and galactosaemia. EFSA Journal 2010;8(9):1777, 29 pp. doi:10.2903/j.efsa.2010.1777
- Hyman PE, Milla PJ, Benninga MA, Davidson GP, Fleisher DF and Taminiau J, 2006. Childhood functional gastrointestinal disorders: neonate/toddler. Gastroenterology, 130, 1519–1526.
- Jones B and Kenward MG, 1989. Design and analysis of cross-over trials. Chapman and Hall, London, United Kingdom.
- Kanabar D, Randhawa M and Clayton P, 2001. Improvement of symptoms in infant colic following reduction of lactose load with lactase. Journal of Human Nutrition and Dietetics, 14, 359–363. Erratum in 2007. Journal of Human Nutrition and Dietetics, 20, 509.
- Kearney PJ, Malone AJ, Hayes T, Cole M and Hyland M, 1998. A trial of lactase in the management of infant colic. Journal of Human Nutrition and Dietetics, 11, 281–285.
- Koch GG, 1972. The use of non-parametric methods in the statistical analysis of the two-period change-over design. Biometrics, 28, 577–584.
- Shamir R, St James-Roberts I, Di Lorenzo C, Burns AJ, Thapar N, Indrio F, Riezzo G, Raimondi F, Di Mauro A, Francavilla R, Leuchter RH, Darque A, Hüppi PS, Heine RG, Bellaïche M, Levy M, Jung C, Alvarez M and Hovish K, 2013. Infant crying, colic, and gastrointestinal discomfort in early childhood: a review of the evidence and most plausible mechanisms. Journal of Pediatric Gastroenterology and Nutrition, 57 (Suppl. 1), S1–S45.

ABBREVIATIONS

AUC	area under the curve
IQR	interquartile range
ITT	intention-to-treat
PP	per-protocol
ppm	part per million